The Examiner rejects claims 1-13 under 35 U.S.C. §112, second paragraph, as being indefinite. The objects to the term "selectively". By the accompanying amendment, this term has been deleted from the claims.

The Examiner rejects claims 1-8 under 35 U.S.C. §102(b) as being anticipated by Bussey, U.S. Patent No. 6,011,148. The Examiner also rejects claim 13 under 35 U.S.C. §103(a) as being unpatentable over Bussey et al. The Examiner states that Bussey et al. teach selective recovery of nucleic acids from liquids, diluting the sample and using an ultrafiltration membrane.

Bussey et al. '148 disclose using tangential flow filtration with an ultrafiltration membrane to purify pharmaceutical grade DNA. Sample is circulated through an ultrafiltration unit until a gel layer forms. The gel layer is then filtered and the nucleic acid is collected as the retentate. Bussey et al. do not address fractionating nucleic acids, but rather purifying nucleic acids from other components. Dilution in Bussey et al. relates to contaminant removal and increased solute purity, not to increase recovery of nucleic acid solute as in the present invention.

By the accompanying amendment, claims 1 and 6 have been amended to recite a process for the fractionation of linear nucleic acids, and to recite that the diluted sample is filtered to dryness. Support for fractionation can be found at page 2,

last paragraph of the specification. Support for filtering to dryness can be found in the Examples and Figures. Bussey et al. do not fractionate the DNA. In addition, Bussey et al. teach away from filtering to dryness. Specifically, Bussey et al. teach diafiltration, which is a tangential flow filtration process wherein buffer is introduced while filtrate is removed. Thus, by definition, the sample in Bussey et al. is not filtered to dryness as is now recited in claim 1 as amended.

With respect to claim 13, the "pressure differentials" mentioned by Bussey et al. relate to tangential flow filtration, which is very different from the method of the present invention. Those skilled in the art appreciate that tangential flow filtration prevents retained components from building up at the surface of the membrane by the tangential flow of the sample. In addition, the statement in Bussey et al. relied upon by the Examiner as the basis for the suggestion of using a first and then a second pressure is a result of potential shearing of the nucleic acid. Those skilled in the art appreciate that in the instant process for the fractionation of linear nucleic acids, the sizes of nucleic acids of concern are in a range that is not subject to mechanical shear forces.

The Examiner rejects claims 9-12 under 35 U.S.C. §103(a) as being unpatentable over Bussey et al. in view of Simon, U.S. Patent No. 5,434,048. The Examiner admits that Bussey et al.

fail to disclose the use of condensing agents such as bivalent cations, and cites Simon as disclosing monovalent and bivalent cations for removal of contaminants by centrifugal ultrafiltration. The Examiner concludes that it would have been obvious to use the Simon cations in the Bussey process.

The rejection is respectfully traversed.

Bussey et al. teach the use of monovalent cations only as part of a bacterial cell lysis procedure, not as they relate to the fractionation of linear acids in ultrafiltration. Moreover, Simon teaches KCl and $MgCl_2$ as they relate to a typical PCR reaction, again incidental to the purification rather than actually influencing nucleic acid recovery in the purification. In addition, Simon teaches nucleic acid purification using centrifugal ultrafiltration, which is very different from ultrafiltration using pressure differential as recited in claim 9, and tangential flow filtration as used in Bussey et al. Specifically, low concentration factors sometimes delivered by centrifugal ultrafiltration requires the use of multiple diltuion and filtration steps (i.e., diafiltration) to deliver the retentate in a concentrated and sufficiently pure and relatively salt-free form. This is particularly true for small volumne devices because of the low head height associated with these small volumes which results in low driving pressure. Moreover, in the centrifugal process, the pressure does not remain

constant; it is continuously decreasing during the process as the volume and thus the head height decreases during filtration.

New claims 14-19 have been added to further define the invention.

The remaining prior art is believed to have been properly not relied upon in rejecting any claim.

Reconsideration and allowance are respectfully requested in view of the foregoing amendment and remarks.

Respectfully submitted,

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Version With Markings to Show Changes Made

1. (Amended) A process for the [selective recovery] fractionation of linear nucleic acids contained in a liquid sample, comprising diluting said liquid sample, contacting the diluted sample with an ultrafiltration membrane, and subjecting said diluted sample to a pressure differential to filter said diluted sample to dryness.

6. (Amended) A process for the [selective recovery] fractionation of linear nucleic acids contained in a liquid sample, comprising providing an ultrafiltration membrane having an upstream and a downstream side, diluting said sample, and contacting said membrane with said liquid sample, and subjecting said liquid sample to a pressure differential having a pressure less than 25 inches Hg to filter said diluted sample to dryness.

9. (Amended) A process for the [selective removal] fractionation of contaminants in a liquid sample, comprising increasing the concentration of said contaminants by adding to said sample a member selected from the group consisting of nucleic acid condensing agents and monovalent cations, and contacting the sample with an ultrafiltration membrane, and subjecting said sample to a pressure differential.

13. (Amended) A process for the [selective recovery] fractionation of linear nucleic acids contained in a liquid sample, comprising diluting said liquid sample, contacting the

diluted sample with an ultrafiltration membrane, and subjecting said diluted sample to a first pressure, followed by subjecting said diluted sample to a second pressure different from said first pressure.

14. (Newly added) A process for the fractionation of linear nucleic acids contained in a liquid sample, comprising:

providing an ultrafiltration membrane having a predetermined molecular weight limit;

providing said liquid sample wherein said linear nucleic acids comprise nucleic acids having a molecular weight below said predetermined molecular weight limit of said membrane;

diluting said liquid sample; and

filtering said diluted sample with said ultrafiltration membrane by subjecting said diluted sample to a pressure differential, whereby nucleic acids below said predetermined molecular weight limit that absent said dilution would pass through said membrane are retained by said membrane.

15. (Newly added) The process of claim 14, wherein said dilution step dilutes said liquid sample to 1/3 to 1/5 its initial concentration.

16. (Newly added) The process of claim 14, wherein said pressure differential is a constant pressure differential.

17. (Newly added) In a process for the fractionation of linear nucleic acids contained in a liquid sample, in which said

liquid sample is subjected to ultrafiltration whereby nucleic acids having a predetermined number of base pairs normally pass through said ultrafiltration membrane using pressure differential as a driving force, the improvement comprising diluting said liquid sample prior to ultrafiltration in order to retain on said membrane said nucleic acids having said predetermined number of base pairs.

18. (Newly added) The process of claim 17, wherein said predetermined number of base pairs is 300 or less.

19. (Newly added) The process of claim 17, wherein said dilution is carried out with a member selected from the group consisting of water, EDTA, trishydrochloride, a mixture of trishydrochloride and sodium EDTA, and trisethylenediaminetriacetic acid.

Replacement Sheets

1. (Amended) A process for the fractionation of linear nucleic acids contained in a liquid sample, comprising diluting said liquid sample, contacting the diluted sample with an ultrafiltration membrane, and subjecting said diluted sample to a pressure differential to filter said diluted sample to dryness.

6. (Amended) A process for the fractionation of linear nucleic acids contained in a liquid sample, comprising providing an ultrafiltration membrane having an upstream and a downstream side, diluting said sample, and contacting said membrane with said liquid sample, and subjecting said liquid sample to a pressure differential having a pressure less than 25 inches Hg to filter said diluted sample to dryness.

9. (Amended) A process for the fractionation of contaminants in a liquid sample, comprising increasing the concentration of said contaminants by adding to said sample a member selected from the group consisting of nucleic acid condensing agents and monovalent cations, and contacting the sample with an ultrafiltration membrane, and subjecting said sample to a pressure differential.

13. (Amended) A process for the fractionation of linear nucleic acids contained in a liquid sample, comprising diluting said liquid sample, contacting the diluted sample with an ultrafiltration membrane, and subjecting said diluted sample to a

first pressure, followed by subjecting said diluted sample to a second pressure different from said first pressure.

14. (Newly added) A process for the fractionation of linear nucleic acids contained in a liquid sample, comprising:

providing an ultrafiltration membrane having a predetermined molecular weight limit;

providing said liquid sample wherein said linear nucleic acids comprise nucleic acids having a molecular weight below said predetermined molecular weight limit of said membrane;

diluting said liquid sample; and

filtering said diluted sample with said ultrafiltration membrane by subjecting said diluted sample to a pressure differential, whereby nucleic acids below said predetermined molecular weight limit that absent said dilution would pass through said membrane are retained by said membrane.

15. (Newly added) The process of claim 14, wherein said dilution step dilutes said liquid sample to 1/3 to 1/5 its initial concentration.

16. (Newly added) The process of claim 14, wherein said pressure differential is a constant pressure differential.

17. (Newly added) In a process for the fractionation of linear nucleic acids contained in a liquid sample, in which said liquid sample is subjected to ultrafiltration whereby nucleic acids having a predetermined number of base pairs normally pass

through said ultrafiltration membrane using pressure differential as a driving force, the improvement comprising diluting said liquid sample prior to ultrafiltration in order to retain on said membrane said nucleic acids having said predetermined number of base pairs.

18. (Newly added) The process of claim 17, wherein said predetermined number of base pairs is 300 or less.

19. (Newly added) The process of claim 17, wherein said dilution is carried out with a member selected from the group consisting of water, EDTA, trishydrochloride, a mixture of trishydrochloride and sodium EDTA, and trisethylenediaminetriacetic acid.